

511 047  
Rec'd PCT/PTO 12 OCT 2004

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
16 October 2003 (16.10.2003)

PCT

(10) International Publication Number  
WO 03/085139 A1

(51) International Patent Classification<sup>7</sup>: C13K 1/00, 1/08, 3/00, 7/00, 11/00, 13/00, C13D 1/00, 3/00, 3/14, C07H 1/06, 3/00

(21) International Application Number: PCT/EP03/01091

(22) International Filing Date: 4 February 2003 (04.02.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data: 20020675 9 April 2002 (09.04.2002) FI

(71) Applicant (for all designated States except US): DANISCO SWEETENERS OY [FI/FI]; Keilaranta 9, FIN-02151 Espoo (FI).

(72) Inventors; and

(75) Inventors/Applicants (for US only): RAVANKO, Vili [FI/US]; 542 Breezy Point Drive, Clinton, IA 52732 (US). MÄYRÄ, Nina [FI/FI]; Tammitie 20 A 17, FIN-00330 Helsinki (FI). HEIKKILÄ, Heikki [FI/FI]; Ristiniemtie 32 G 33, FIN-02320 Espoo (FI). KOIVIKKO, Hannu [FI/FI]; Kolsarintie 4, FIN-02460 Kantvik (FI). KEKKI, Pekka [FI/FI]; Säterinkatu 5, FIN-30100 Forssa (FI). KALLIOMÄKI, Hannu [FI/FI]; Säterinkatu 3, FIN-30100 Forssa (FI). TYLLI, Matti [FI/FI]; Ristikalliontie 19, FIN-02460 Kantvik (FI). NYGRÉN,

(74) Agents: HOFFMANN EITLE et al.; Arabellastrasse 4, 81925 München (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 03/085139 A1

(54) Title: PURIFICATION OF PURE DISACCHARIDE SOLUTION

(57) Abstract: The present invention pertains to a chromatographic process for separating saccharide monomers from dimers and/or saccharide trimers from dimers. The separation is effected with a cation exchange resin. If saccharide monomers are separated from saccharide dimers the cation exchange resin has a high degree of crosslinking. If the saccharide dimers are separated from saccharide trimers the cation exchange resins has a low degree of crosslinking.

## PURIFICATION OF PURE DISACCHARIDE SOLUTION

The present invention pertains to a chromatographic process for separating saccharide monomers from saccharide dimers and/or for separating saccharide trimers from saccharide dimers.

Saccharides in the context of the present invention may either be sugars or sugar derived alcohols. Such saccharides, and in particular disaccharides such as maltose and maltitol, have recently attracted increased attention as advantageous sweetening agents for various food stuffs, as well as for other applications.

To date, a number of processes for producing saccharides, and in particular disaccharides, are known. For example, processes for producing maltose syrups, pure maltose as well as maltitol syrups and pure maltitol, are known. The common denominator of these processes is that they use starch as the starting material. Commonly, starch of various origins is liquefied and the thus obtained liquefied starch is treated with various enzymes so as to produce a maltose containing product.

Depending on the process employed, these maltose containing products vary significantly not only in their maltose content but also in view of the amount of maltotriose and glucose contained therein.

In order to arrive at a useful maltose containing product, the known processes proposed various purification procedures such as crystallisation or chromatographic separation. Membrane separation processes have also been proposed. The

products obtained after these purifications may then be used as they are obtained, or subjected to further treatment. For example, maltose containing products may, after ion exchange, be hydrogenated so as to obtain maltitol containing products. Depending on the purity these products must also be purified further.

US 4,487,198, for example, discloses a process for purifying maltose syrup from a feed containing at least 70 wt%-DS maltose (wt%-DS as used in this description, and the claims means wt% on a dry solids basis) in addition to glucose and dextrin by way of a chromatographic separation. More precisely this process uses a strongly acidic cation exchange resin having sulphonyl groups of an alkaline metal or alkaline earth metal form to generate five fractions from the feed material.

The first fraction is rich in dextrin, the second fraction contains dextrin and maltose, the third fraction is rich in maltose, the fourth fraction contains maltose and glucose and the fifth fraction contains glucose. The mixed fractions, i.e. the second and fourth fractions are returned into the column in order to eventually obtain a high maltose syrup.

A further process for purifying maltose containing feed solutions by way of chromatography is disclosed in US 4,970,002. Processes leading to crystalline maltose are disclosed in US 5,112,407 and US 4,816,445.

US 5,462,864 discloses a process for producing high purity maltitol, comprising the steps of liquefying starch, saccharifying the liquefied starch and reducing the product mixture obtained in the saccharification step in order to obtain a maltitol containing product. This US patent also contemplates a chromatographic purification of both the maltose containing product as well as the maltitol containing product.

A similar process for producing maltitol is disclosed in US 4,846,139. US 4,846,139 discloses a process for the preparation of crystalline maltitol comprising successively: catalytic hydrogenation of a saccharified starch milk, a step of chromatographic fractionation of the hydrogenated syrup, crystallisation and separation of the maltitol crystals and recycling the mother liquor of the crystallisation into the fractionation step.

While the aforementioned techniques may have their merits, there still remains a need for further improvement. In particular, there is a strong need to increase the maltose content especially in view of the separation of the maltose from glucose and maltotriose. In fact, there is a general need for improvement in the separation of disaccharides from mono- and trisaccharides.

This separation is rather difficult according to known methods: sugar dimers such as maltose, sugar monomers such as glucose and sugar trimers such as maltotriose have rather similar retention times in prior art separation columns. In fact, this is not only true for sugar monomers, dimers and trimers, but also for saccharide monomers (like sugar alcohols), dimers and trimers in general.

Because of the similar retention times in prior art chromatographic separations one had to compromise on the yield in order to obtain a good purity or visa versa. In some circumstances however, this compromise has lead to less than satisfying results, as high demands on purity entailed very low yields and thus rather uneconomic processes.

A good example for this is the production of crystalline maltitol. Maltotritol, i.e. the hydrogenation product of maltotriose, inhibits the crystallisation of maltitol even in small quantities. Accordingly, it is very difficult to obtain crystalline maltitol in satisfying quantities in the presence of Maltotritol. Maltotritol or its precursor the

maltotriose must therefore be removed to the largest possible extent. Similar difficulties have been found when crystallizing sucrose in the presence of raffinose (sugar beet process) or in the presence of kestoses (cane sugar process). The same problem exists also in the cellulose and hemicellulose hydrolyzates, which contain saccharides with various chain lengths as degradation products. Therefore, the need to remove trimers and monomers from dimer solutions exists in general.

In light of the above, the present invention therefore aims at providing an improved process for separating saccharide dimers from monomers, and saccharide trimers from dimers so as to obtain saccharide monomers, dimers and/or trimers with high purity in an economical process.

The process set forth in the claims solves this object. The process is based on the recognition that, depending on the saccharides to be separated, the degree of crosslinking of the ion exchange resin must be adjusted. Ion Exchange resin is preferably a gel type cation exchange resin, and most preferably a gel type strong acid cation exchange resin.

The process is a column separation method, where the column filling material is ion exchange resin. The ion exchange resin can be chosen from a cation exchange resins (with styrene or acryl skeleton) as strong acid cation exchange resins or weak acid cation exchange resins.

The improvement achieved by the present invention resides, in particular, in the effective separation of disaccharides or saccharide dimers from saccharide monomers, and the separation of saccharide dimers from trimers. At the same time the process according to the present invention avoids unnecessary dilution of the product fractions and thereby achieves the aforementioned advantages without sacrificing the overall performance, i.e. purity and yield. Further

advantages of the present invention will be apparent from the following detailed description.

The above object is achieved by a chromatographic process. This process may be designed as a sequential simulated moving bed process, a continuous simulated moving bed process, a batch chromatographic process or variants and combinations of these.

In this process a feed solution is subjected to a chromatographic separation with the aid of a crosslinked ion exchange resin, which is preferably a gel type cation exchange resin and most preferably a gel type strong acid cation exchange resin. If the composition of the feed solution is such that saccharide monomers should be separated from saccharide dimers, then the degree of crosslinking in the cation exchange resin should be high. If the composition of the feed is such that saccharides dimers should be separated from saccharides trimers, then the degree of crosslinking of the cation exchange resin should be low.

It should thereby be understood that a separation of saccharide monomers from saccharides dimers can lead to a saccharide monomer product fraction and a mixed fraction containing saccharide dimers. It can also lead to a mixed fraction and a saccharide dimer product fraction. Such a separation can also lead to two product fractions, namely a monosaccharide product fraction and a saccharide dimer product fraction. The same considerations apply mutatis mutandis to separating saccharide dimers and saccharides trimers. It is of course, also possible to treat a feed solution comprising saccharide monomers, dimers and trimers by separating the saccharide monomers from a mixed fraction in a first step and then separating the dimers from the mixed fraction in a second step. Likewise, it is also possible to purify such a monomer, dimer and trimer mixture by first of all separating a trimer fraction from a mixed monomer and

dimer fraction, and to fractionate the mixed monomer and dimer fraction in a second step.

A resin with a high degree of crosslinking is preferably a resin that has a degree of crosslinking of 5 to 8 %. A resin with a low degree of crosslinking preferably has a degree of crosslinking of 2 to 4.5 %. The term 'degree of crosslinking' as used herein is defined in accordance with H.-G. Elias, *Macromoleküle*, Hüthig & Wepf Verlag Basel : Heidelberg and New York, 4<sup>th</sup> edition, 1981.

According to this definition the degree of crosslinking means the weight ratio of the crosslinkable monomers to the total monomers. It is conveniently expressed in percent.

It has surprisingly been found that the previously difficult separations of saccharide monomers from saccharide dimers, and saccharide dimers from saccharide trimers can be improved dramatically when choosing a crosslinked ion exchange resin with a particular degree of crosslinking, which is preferably a gel type cation exchange resin and most preferably a gel type strong acid cation exchange resin.

That is, the separation of monomers from dimers can be made effectively when using a ion exchange resin with a high degree of crosslinking, preferably a degree of crosslinking of 5 to 8 %. At the same time, saccharide dimers can be separated effectively from saccharide trimers with a ion exchange resin having a low degree of crosslinking, preferably 2 to 4.5 %. Typically, such separations remove trisaccharides by at least 75% and monomers by at least 65%. In the same context in a special embodiment the yield of dimers is over 85 %.

It is thus a particular advantage of the process according to the present invention that the use of a particular crosslinked cation exchange resin for a particular starting material, i.e. a particular separation purpose, leads to very

favourable separations. The present invention thereby overcomes the difficulties encountered in the prior art methods. This is true both for the separation of saccharides such as sugar monomers, dimers, and trimers, as well as saccharides such as sugar alcohol monomers, dimers and trimers.

The feed solution for the process according to the present invention may be any solution containing saccharide monomers, dimers and/or trimers. Usually the saccharide dimer will be the major component and preferably be present in an amount of 65 to 85 wt%-DS (i.e. weight-% on dry substance) in the feed solution. The amount of saccharide monomers and/or trimers contained in the feed solution is not particularly limited. However, it is preferred if they are present in an amount that is less than that of the saccharide dimer.

The method according to the present invention is particularly useful for separating feed solutions containing a large amount of saccharide dimer, such as 65 to 85 wt% - DS and only minor amounts of saccharide monomers and/or trimers. The method is useful when the amount of saccharide monomers and/or trimers is less than 10 wt%-DS and it is particularly useful when the amount of saccharide monomers and/or trimers is 3 wt%-DS or less, preferably 2 wt%-DS or less and in particular 1.5 wt%-DS or less.

The feed solution may contain various solvents such as ethanol and/or water. However, aqueous solutions are preferred.

Typical feeds for obtaining maltose rich fractions are feed solutions obtained as the result of starch hydrolysis. For the purpose of the present invention, it is not critical what kind of starch is subjected to hydrolysis and both amylase rich and amylopectin rich starches can be used. Common sources for such starches are potatoes, barley, corn, rice,

sago, tapioca and other natural products well known in the art.

In order to produce a starch hydrolysis one typically in a first step liquefies or gelatinises a starch slurry. This may be done according to methods well known in the art. For example, such liquefaction can be achieved by using liquefying enzymes, acids or simply by heating the starch slurry to elevated temperatures.

The effect of the liquefaction step is the fragmentation of the starch molecule. The resulting fragments are then degraded further with the aid of enzymes in the subsequent saccharification step. The saccharification step, which may also be carried out according to methods well known in the art, leads to a mixture comprising maltose, glucose, maltotriose and other polydextrins.

The saccharification is also typically effected enzymatically. For this purpose, the use of  $\beta$ -amylases and 1.6 glycosidase such as isoamylase and pullulanase has proven successful. The saccharification is also well known in the art (Starch: Chemistry and Technology Academic Press, 1984).

One embodiment to utilize the invention is to produce starch hydrolyzate with enzymes in a way, that maltose content is high but the content of impurities like trimers (e.g. maltotriose) and monomers (e.g. glucose) are in the low level. The chromatographic separation method of the invention is used to remove the impurity, which exists in the higher concentration by choosing the resin with the relevant crosslinking degree.

Another embodiment to utilize the invention is to apply the separation method to the hydrolyzate solution of cellulose and hemicellulose; cellulose hydrolyzate comprising glucose, cellobiose and cellotriose and hemicellulose hydrolyzate

comprising e.g. xylose, xylobiose and xylotriose or other hemicellulose based monomers, dimers and trimers.

The method of the invention can be applied also for the separation of sugar molasses. In case of the sugar beet molasses the saccharides are glucose, fructose as monomers, sucrose as dimer and raffinose as trimer. In case of cane sugar molasses monomers are glucose and fructose, dimer is sucrose and trimers are kestoses.

For the purpose of the present invention it was found that it is particularly advantageous if the liquefied starch is treated as follows.

In a first step the liquefied starch is saccharified with pullulanase (e.g. Optimax<sup>®</sup> Optimalt<sup>®</sup>, dosage typically 1 l/ton - DS) and  $\beta$ -amylase (e.g. BBA<sup>®</sup> dosage typically 1 l/t - DS). It is also particularly advantageous if subsequently some low temperature  $\alpha$  - amylase (e.g. BAN<sup>®</sup> dosage typically 0.01 l/t-DS) is added and a final saccharification is achieved by way of the addition of maltogenic  $\alpha$  - amylase (e.g. Maltogenase<sup>®</sup> dosage typically 1.5 l/t-DS). The incubation times between enzyme additions are typically 0 hour to 20 hours depending on the speed of the added enzyme.

Effecting the saccharification by way of sequential addition of the enzymes allows to avoid maltose losses. The simultaneous addition of enzymes often leads to excess production of glucose and/or maltotriose. It is especially favourable to delay the addition of low temperature  $\alpha$  - amylase as this enzymes exhibits random endo-activity. Prolonged action of this enzymes leads to the formation of uneven starch chain ends, and thus to an increased formation of maltotriose as the  $\beta$  - amylase action will be stopped more often to form uneven chain ends.

Proceeding in the above described way is particular advantageous as it yields a maltose syrup with a maltose

content of approx. 88 wt%-DS or more. At the same time, the maltotriose content is 0.9 wt%-DS or less. However, it must also be noted that reaching very low maltotriose levels requires more enzyme or a longer process time. In practice, it may therefore be more advantageous to compromise the maltotriose concentration rather than to increase the costs, by using more enzymes or allowing for a prolonged hydrolysis time.

It should also be noted that depending on the type of starch and the degree of liquefaction of the raw material, it may be advantageous to add pullulanase  $\beta$  -amylase and maltogenic  $\alpha$ -amylase sequentially rather than simultaneously.

Generally speaking, it is favourable for the process according to the present invention to use a feed solution for the chromatographic separation with a dry substance content of 25 to 70 wt%, especially 35 to 55 wt%. However, the process according to the present invention is not particularly limited in this respect.

It is also advantageous to use a feed solution with a disaccharide content of more than 70 wt%-DS and in particular from 75 wt% to 90 wt% - DS, or even better 75 wt% to 85 wt%-DS. However, feed solutions with disaccharide contents outside these ranges can also be used.

The feed solution as described above is then subjected to the chromatographic process according to the present invention. As mentioned before, the chromatographic separation according to the present invention uses a crosslinked ion exchange resin preferably gel type cation exchange resin. This crosslinked cation exchange resin may either be a strong or a weak acid cation exchange resin with styrene or acrylic skeleton.

Weakly acidic cation exchange resins may favourably be used e.g. for the separation of more hydrophobic saccharides.

Weakly acid cation exchange resins are particularly useful for feeds containing hydrophobic monosaccharides such as deoxy, methyl and anhydro sugars, as well as sugar alcohols from more hydrophilic sugars.

Most preferably, the weakly acidic cation exchange resin is used for separating saccharides such as hexoses, including ketohexoses, aldohexoses, pentoses such as ketopentoses aldopentoses, corresponding sugars and sugar alcohols as well as mixtures thereof, e.g. glucose, fructose, rhamnose anhydrosorbitol, sorbitol, erythritol, inositol, arabinose, xylose and xylitol. Sucrose, betaine and amino acid containing solutions can also be separated advantageously. The weakly acid cation exchange resin can also be used for separating anhydrosugars from corresponding sugars as well as anhydrox sugar alcohols from corresponding sugar alcohol.

Preferably, the weakly acid ionic exchange resin is a crosslinked acrylic resin with carboxylic functional groups for example Finex CA 16 GC (8% DVB). The resin may be in the H<sup>+</sup>, K<sup>+</sup>, Na<sup>+</sup>, Mg<sup>2+</sup>, or Ca<sup>2+</sup> form. It may also be used in other forms.

For the most part however, one will use strong acid cation exchange resins for the separation of the saccharides. Strong acid cation exchange resins on the basis of sulphonated styrene divinyl benzene copolymers are particularly useful in the context of the present invention. Examples of such resin are Finex CS 8 GC (4% DVB, particle size 0.36 mm, manufactured by Finex Ltd., Finland) Purolite PCR 664 (6.5% DVB, particle size 0.4 mm, manufactured by Purolite Co., USA) and Amberlite C 3120 (6% DVB, particle size 0.35mm, manufactured by Rohm and Haas, USA).

These resins are advantageously used in their alkaline metal or earth alkaline metal form, whereby the alkaline metal form

should be understood so as to include the  $\text{NH}_4^+$  form as well. Typically, resins in the  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  forms are preferred.

As far as the amount of sulphonyl groups in the strongly acid cation exchange resins is concerned, it should be noted that usually the sulphonation of these styrene rings is close to 100%. However, lesser degrees of sulphonation can also be used within the context of the present invention, provided that such resins still allow the above object to be achieved.

The aforementioned resins are packed in a column which is loaded with the feed solution. Typically, a feed solution containing 20 to 80wt%-DS is loaded onto the column in an amount of 5 to 20 vol.% based on the volume of the column.

The temperature at which the process according to the present invention is performed is not particularly limited. However, it has been found that elevated temperatures such as temperatures of 60°C or more lead to better results. Particularly good results are obtained at temperatures of 75°C or more.

As the feed solutions according to the present invention are preferably aqueous feed solutions, it is also generally favourable to work at temperatures below 100°C. The best temperature for performing the chromatographic separation according to the present invention thus falls within the range of 65 to 90°C. For maltose and maltitol containing syrups the preferred temperature is 80°C or higher.

As mentioned before, the process according to the present invention may lead to fractions rich in saccharide monomers, dimers and/or trimers. The product fractions and in particular the saccharide dimer product fractions are thereby of particularly high purity. That is such fractions usually contain 90 to 96 wt%-DS or more product, e.g. disaccharide. At the same time, the amount of impurities e.g. saccharide

monomers and/or trimers in a disaccharide product fraction, is extremely small.

In as much as the present invention pertains to product fractions, it should be borne in mind that this means a single fraction or a mixture of 2 or more fractions rich in the respective product. It may also mean fractions from consecutive feeds as well as fractions derived from fractions obtained in the chromatographic separation or membrane separation process e.g. by way of concentration.

In the present invention resins different cross-linking can be used in columns, which are operated in parallel or in series. Part of the resin beds in parallel or serial columns can consist of resin with high or low crosslinking.

The following examples illustrate the invention. It should be borne in mind that the procedures, individual process steps, conditions and numerical values indicated in these examples are representative of the present invention and should not be construed as limited to the specific context of the individual example only.

## EXAMPLES

**Example 1** - Preparation of a low maltotriose feed solution by adding the enzymes simultaneously

A hot (temp > 65°C) liquefied starch solution from starch liquefying process having a DE of 7,8 and a dry solids content of 25 wt% was adjusted to a pH of 5,5 and cooled to 58°C. At this temperature four enzymes 1,5 l/t dry solids (DS) Maltogenase™ 4000 L, 1 l/t-DS Promozyme® 600 L, 1 l/t-DS beta-amylase Optimalt® BBA and 0,01 l/t-DS BAN 240L were added simultaneously = 0 h. The composition of the solution changed as follows:

Time	Oligo	Maltotriose	Maltose	Glucose	Sum
h	wt%- DS	wt%- DS	wt%- DS	wt%- DS	wt%- DS
2	24,71	7,50	61,49	1,44	95,14
18	12,74	3,68	77,79	4,39	98,61
26	10,19	2,65	80,46	5,08	98,38
48	7,56	1,64	83,42	5,91	98,52
68	5,81	0,92	85,33	6,34	98,39

This solution can be subjected to the chromatographic separation with high DVB-resin in order to remove glucose.

**Example 2** - Low maltotriose feed solution by delayed Addition of low temperature a-amylase

Example 1 was repeated. However, the temperature was set to 60°C and the dry matter content was increased from 25 to 30wt%. In addition, a different pullulanase, Optimax L-1000 (Genencor) was used and Novo BAN 240 L (low

temperature -  $\alpha$ -amylase) was added after 50 h from start of saccharification. The details and results are summarised in the table below.

Enzyme	Time	Oligo	Maltotriose	Maltose	Glucose
0 h :	h	wt%- DS	wt%- DS	wt%- DS	wt%- DS
	2	12,0	7,2	72,2	1,4
	18	6,1	2,8	81,3	3,4
	24	5,8	2,0	82,8	3,8
BAN 240 L	50	4,8	0,9	87,5	4,7
	72	4,7	0,5	85,3	4,9
	144	4,9	0,1	85,8	5,4

This solution can be further treated similar than in Example 1 in order to obtain solution with high maltose purity.

**Example 3 - Low maltotriose feed solution by sequential addition of saccharifying enzymes**

A liquefied barley starch solution having a DE of 4,6 was adjusted to 30wt%-DS and to pH 5,5 before enzyme addition. The temperature of the liquid was adjusted to 60°C and 1 l/t DS Optimax® L-1000 pullulanase (from Genencor) was added. After 23 hours at 60°C 1 l/t DS beta-amylase (Optimalt® BBA from Genencor) was added. After 30 hours 1,5 l/t DS maltogenic alpha-amylase (Maltogenase™ 4000 L from Novo) and 0,01 l/t DS low temperature alpha-amylase (BAN 240 L from Novo) were added. In total the product was incubated for 72 hours at 60°C. The composition developed as follows:

	Maltotriose	Maltose	Glucose	>4 oligomers
h	wt%-DS	wt%-DS	wt%-DS	wt%-DS
24	8,77	68,61	0,38	8,85
48	1,20	88,87	4,21	3,52
72	0,74	90,50	4,78	3,90

This solution is preferably further purified with chromatographic separation method with high DVB-resin to remove glucose.

**Example 4 - Low glucose high maltotriose feed solution**

Liquefied corn starch having DE 10 was cooled to 60°C. The pH of the 25wt%-DS solution was adjusted to 5,5. Then 1 l/t DS Optimax® L-1000 pullulanase (from Genencor) was added. After 24 hours 1 l/t DS Optimalt® BBA beta-amylase (from Genencor) was added. The hydrolysate was incubated for a total of 72 hours at 60°C. The composition developed as follows:

	Maltotriose	Maltose	Glucose	>4 oligomers
h	wt%-DS	wt%-DS	wt%-DS	wt%-DS
2	12,1	61,6	0,5	25,2
18	13,7	70,3	0,6	13,7
24	14,5	72,1	0,5	11,1
48	15,7	75,9	0,6	6,3
72	16,0	77,1	0,6	5,7

The solution is subjected to chromatographic separation with low DVB-resin to remove maltotriose.

**Example 5** - High maltose, low maltotriose feed solution by adding the enzymes sequentially at higher temperatures

Liquefied barley starch of 30wt%-DS with a DE of 2,6 was adjusted to pH 5,5 before enzyme additions. The temperature of the liquid was set to 65°C and a total of 0,4 l/t DS maltogenic alpha-amylase (Maltogenase™ 4000 L from Novo), 1 l/t DS beta-amylase (Optimalt® BBA from Genencor) and 1 l/t DS Optimax® L-1000 pullulanase (from Genencor) were added in four equal lots while stepwise lowering the temperature to 65, 64, 62, 60°C in 40 minutes intervals. After 12 hours 0,01 l/t DS low temperature alpha-amylase (BAN 240 L from Novo) was added. After 24 hours 1,1 l/t DS maltogenic alpha-amylase (Maltogenase™ 4000 L from Novo) was added. In total the product was incubated for 100 hours. The composition developed as follows:

	Maltotriose wt%-DS	Maltose wt%-DS	Glucose wt%-DS	>4 oligomers wt%-DS
h				
2	8,14	81,03	0,83	9,98
16	7,34	84,73	1,56	6,36
24	6,24	85,70	2,12	5,95
48	0,95	87,97	4,69	6,39
100	0,43	87,31	5,02	7,24

The solution can be subjected to chromatographic separation with high DVB-resin to remove glucose.

**Example 6 - Chromatographic separation of maltose solution with high glucose content**

Starch hydrolysate (maltose hydrolysate) was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,6 m was filled with a strong acid cation exchange resin. The height of the resin bed was approximately 5,2 m. The degree of cross-linkage was 5,5 w-% DVB and the average particle size of the resin was 0,35 mm. The resin was regenerated into sodium ( $\text{Na}^+$ ) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 80°C. The flow rate in the column was adjusted to 210 l/h.

Chromatographic separation was carried out as follows:

**Step 1.**

The dry substance of the feed solution was adjusted to 36 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

**Step 2.**

110 l of the preheated feed solution was pumped to the top of the resin bed.

## Step 3.

The feed solution was eluted downwards in the column by feeding preheated deionised water to the top of the column.

## Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into five fractions in the following order: first residual fraction (containing oligosaccharides), recycle fraction (containing mostly maltose and maltotriose), maltose rich fraction (containing most of the maltose), second recycle fraction (containing mostly maltose and glucose) and second residual fraction (containing mostly glucose). Both recycle fractions were combined with the feed solution.

The amount of dry substance as well as maltose content in the feed solution and in product fraction are presented in the table below. The concentration of maltose is expressed as percentage of the total dry substance in the particular fraction. The yield of maltose in product fraction is also presented (the amount of the component in the particular fraction in relation to the total amount of that component in all product fractions excluding recycle fractions). Oligosaccharide, maltotriose and glucose removals are also presented. Removal is expressed as the amount of the component in residual fractions compared to the amount of that component in residual fractions and in product fraction.

	Feed solution	Maltose fraction
DS in fraction, kg	45	25
DS g/100 g solution	36	21
Maltose wt%-DS	82	97
Maltotriose wt%-DS	1,7	0,4
Glucose wt%-DS	6,1	1,9
Oligosaccharides wt%-DS	7,8	0,4
Maltose yield % (with recycle)		97
Oligosaccharide removal %		94
Maltotriose removal %		69
Glucose removal %		62

A resin with 5,5 w-% DVB separated well maltose from other components. Especially, the resin separated well maltose from glucose. Maltose purity was increased by 15%-units. Maltose yield was 97%. Results are shown in Fig. 1.

**Example 7 - Chromatographic separation of maltose solution with high glucose content**

Starch hydrolysate (maltose hydrolysate) was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,225 m was filled with a strong acid cation exchange resin. The height of the resin

bed was approximately 5,2 m. The degree of cross-linkage was 4 w-% DVB and the average particle size of the resin was 0,36 mm. The resin was regenerated into sodium ( $\text{Na}^+$ ) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 80°C. The flow rate in the column was adjusted to 30 l/h.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 36 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

15 l of the preheated feed solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated ion-exchanged water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into five fractions in the following order: first residual fraction (containing oligosaccharides), first recycle fraction (containing mostly maltose and maltotriose), maltose rich fraction (containing most of the maltose), second recycle fraction (containing mostly maltose and glucose) and second residual fraction (containing mostly glucose). First recycle fraction was introduced to the front slope and second recycle fraction to

the back slope of the concentration profile of chromatographic separation.

Yields and composition of solution (maltose purity) are calculated similar than in Example 6.

	Feed solution	Maltose fraction
DS in fraction, kg	6,0	2,8
DS g/100 g solution	36	16
Maltose wt%-DS	83	89
Maltotriose wt%-DS	2,0	0,3
Glucose wt%-DS	6,5	9,0
Oligosaccharides wt%-DS	6,2	0,1
Maltose, yield %		84
Oligosaccharide removal %		99
Maltotriose removal %		92
Glucose removal %		6,1

A resin with 4 w-% DVB separated well maltose from other components. Especially, the resin separated well maltose from oligosaccharides and maltotriose. Maltose purity was increased by 6%-units. Maltose yield was (84%). Results are shown in Fig. 2.

#### Example 8 - Chromatographic separation of fructose run-off

Fructose run-off from fructose crystallization of a process based on sucrose, was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,225 m was filled with a weakly acid cation exchange resin. The height of the resin bed was approximately 5,2 m. The degree of cross-linkage was 8 w-% DVB and the average particle size of the resin was 0,29 mm. The resin was regenerated into sodium ( $\text{Na}^+$ ) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 65°C. The flow rate in the column was adjusted to 30 l/h. The pH of the resin was adjusted to approximately 4,5 by circulating acidic 5% Na-acetate solution through resin.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 30 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

18 l of the preheated feed solution was pumped to the top of the resin bed.

Step 3.

The feed solution was eluted downwards in the column by feeding preheated deionised water to the top of the column.

Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into three fractions in the following order: residual fraction (containing mostly oligo- and disaccharides), recycle fraction (containing mostly glucose and fructose) and fructose rich fraction (containing most of the fructose).

Recycle fraction was combined with the feed solution.

Yields and concentrations of components are calculated similar than in Example 6.

	Feed solution	Fructose fraction
DS in fraction, kg	6	4,2
DS g/100 g solution	30	10
Fructose wt%-DS	92	95
Glucose wt%-DS	2,2	1,6
Oligo- and disaccharides wt%-DS	2,2	0,2
Fructose, yield %		96
Oligo- and disaccharide removal %		97
Glucose removal %		31

A resin with 8 w-% DVB separated well fructose from other components. Especially, the resin separated well fructose from oligo- and disaccharides. Fructose yield was 96%.

**Example 9 - High maltotriose and high glucose feed separations**

Two different maltose hydrolysates were used. One contained more than 1,5wt%-DS maltotriose and the other contained more than 1,5wt%-glucose. The separation was carried out with a 4,0 DVB-% resin and a 5,5 DVB-% resin, respectively. The results are summarised in the table below.

Resin DVB-%	4,0	5,5
<b>Feed composition (wt%-DS)</b>		
Maltose	75,2	83,7
Oligosaccharides	7,3	7,2
Maltotriose	13,6	1,5
Glucose	1,2	6,3
<b>Product composition (wt%-DS)</b>		
Maltose	91,0	91,0
Oligosaccharides	0,0	2,2
Maltotriose	3,1	1,2
Glucose	2,6	2,5
Maltose recovery (%)	90	98,6
Recycle ratio (%)	18,2	0,0
Oligosaccharide removal (%)	100	72,5
Maltotriose removal (%)	70,4	24,8
Glucose removal (%)	6,5	60,4

**Example 10 - Purification and hydrogenation of maltose separation product**

The maltose product from chromatographic separation process was purified using ion exchange as a tool. The resins in the purification step were strong acid cation exchange resin and weak base anion exchange resin. The temperature during the purification was 60 degrees of centigrade and flow through the resins was two bed volumes in hour. Feed syrup dry substance content was 50 %.

The hydrogenation was made in mixed batch autoclave at temperature of 115 degrees of centigrade and at 40 bar pressure using Raney nickel as a catalyst. The catalyst load was 10 % wet catalyst of batch dry substance. The hydrogenation time was four hours.

**Example 11 - Chromatographic separation of maltitol**

Commercial maltitol syrup was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,225 m was filled with a strong acid cation exchange resin. The height of the resin bed was approximately 5,2 m. The degree of cross-linkage was 4 w-% DVB and the average particle size of the resin was 0,36 mm. The resin was regenerated into sodium ( $\text{Na}^+$ ) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 80°C. The flow rate in the column was adjusted to 30 l/h.

Chromatographic separation was carried out as follows:

**Step 1.**

The dry substance of the feed solution was adjusted to 36 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

**Step 2.**

15 l of the preheated feed solution was pumped to the top of the resin bed.

**Step 3.**

The feed solution was eluted downwards in the column by feeding preheated deionised water to the top of the column.

## Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into five fractions in the following order: first residual fraction (containing oligosaccharides), recycle fraction (containing mostly maltitol and maltotritol), maltitol rich fraction (containing most of the maltitol), second recycle fraction (containing mostly maltitol and sorbitol) and second residual fraction (containing mostly sorbitol). Both recycle fractions were combined with the feed solution.

Yields and concentrations of components are calculated equally than in Example 6.

	Feed solution	Maltitol fraction
DS in fraction, kg	6	2,7
DS g/100 g solution	36	16
Maltitol wt%-DS	63	94
Maltotritol wt%-DS	15	2,7
Sorbitol wt%-DS	3,9	0,8
Oligosaccharides wt%-DS	15	0,1
Maltitol, yield %		89
Oligosaccharide removal %		100
Maltotritol removal %		89
Sorbitol removal %		88

A resin with 4 w-% DVB separated well maltitol from other components. Especially, the resin separated well maltitol from oligosaccharides and maltotritol. Maltitol purity was increased by 31%-units. Maltitol yield was 89%.

**Example 12 - Chromatographic separation of maltitol run-off, solution with low sorbitol content**

Maltitol run-off from maltitol crystallization was subjected to a chromatographic separation in a batch separation column. The separation was performed in a pilot scale chromatographic separation column as a batch process.

The whole equipment consisted of a feed tank, a feed pump, a heat exchanger, a chromatographic separation column, product containers, pipelines for input of feed solution as well as eluent water, pipelines for output and flow control for the outcoming liquid.

The column with a diameter of 0,225 m was filled with a strong acid cation exchange resin. The height of the resin bed was approximately 5,2 m. The degree of cross-linkage was 4 w% DVB and the average particle size of the resin was 0,36 mm. The resin was regenerated into sodium ( $Na^+$ ) form and a feeding device was placed at the top of the resin bed. The temperature of the column, feed solution and eluent water was 80°C. The flow rate in the column was adjusted to 30 l/h.

Chromatographic separation was carried out as follows:

Step 1.

The dry substance of the feed solution was adjusted to 36 g dry substance in 100 g of solution according to the refractive index (RI) of the solution.

Step 2.

9 liters of the preheated feed solution was pumped to the top of the resin bed.

## Step 3.

The feed solution was eluted downwards in the column by feeding preheated deionised water to the top of the column.

## Step 4.

The density and conductivity of the outcoming solution were measured continuously. The outcoming solution was collected and divided into five fractions in the following order: first residual fraction (containing oligosaccharides), recycle fraction (containing mostly maltitol and maltotritol), maltitol rich fraction (containing most of the maltitol), second recycle fraction (containing mostly maltitol and sorbitol) and second residual fraction (containing mostly sorbitol). Both recycle fractions were combined with the feed solution.

Yields and concentration of components have been calculated equally than in Example 6.

	Feed solution	Maltitol fraction
DS in fraction, kg	3,8	2,3
DS g/100 g solution	36	15
Maltitol wt%-DS	91	96
Maltotritol wt%-DS	6	0,6
Sorbitol wt%-DS	0,9	0,6
Oligosaccharides wt%-DS	0	0
Maltitol, yield %		93
Maltotritol removal %		91
Sorbitol removal %		67

A resin with 4 w-% DVB separated well maltitol from other components. Especially, the resin separated well maltitol from maltotritol. Maltitol purity was increased by 5%-units. Maltitol yield was 93%.

**Example 13 - Maltitol crystallization**

A crystallization test was made by using about 213 kg of maltitol feed syrup with purity 93,3 % and a dry substance concentration of about 63,2 wt.-%. The solution contained also sorbitol 3,0 wt%-DS, maltose 0,1wt%-DS and maltotriol 0,4wt%-DS.

The solution was continuously fed into a 400 liter evaporative vacuum crystallizer (boiling pan) where it was agitated and concentrated under reduced pressure by boiling. The liquid level was kept low by adjusting the feed rate. The seeding was made by 0,06% milled maltitol crystals at DS 78,2% at 60,2°C (supersaturation about 1,19). After seeding the crystal containing mass was further concentrated and agitated by boiling for 4,3 hours at about 60°C to DS 89,9% and the liquid level was increased at the same time. The temperature was controlled by pressure. Crystal size after boiling crystallization was 50-100µm.

After boiling the mass was divided into two cooling crystallizers. The main part of the mass was mixed at 60°C constant temperature overnight. Crystallization yield was 70 % M/M when calculated from mother liquor sample. Final crystal size after crystallization was 100-200µm.

Centrifugation was made 21 hours from the seeding with a laboratory centrifuge (basket Ø 23cm). The crystal cake assay without wash was 99,7 % DS and crystal yield was 66,9 % DS/DS.

The rest of the boiling crystallized mass was cooled from 60°C to 50°C linearly during 17 hours. The crystallization yield was 76,7 % M/M when calculated from mother liquor sample. The crystal size after cooling was 100-200µm.

Centrifuging was made 28 hours from seeding with the laboratory centrifuge (basket Ø 23 cm). The crystal cake assay without wash was 97,4wt%-DS and the crystal yield was 73,5 % DS/DS. With 10% wash the assay of the cake was 98,6wt%-DS and yield 62,2wt% DS/DS.

## CLAIMS

1. Chromatographic process for separating saccharide monomers from dimers and/or saccharide trimers from dimers, wherein an ion exchange resin with a high degree of crosslinking is used when saccharide monomers are separated from dimers and a ion exchange resin with a low degree of crosslinking is used when saccharide trimers are separated from dimers.
2. Process according to claim 1 wherein the resin for separating saccharide monomers from dimers has a degree of crosslinking of 5 to 8 % and the resin for separating saccharide trimers from dimers has a degree of crosslinking of 2 to 4.5%.
3. Process according to claim 1 or claim 2 wherein the feed solution contains a saccharide dimers and 2wt%-DS or less of a saccharide monomer and/or saccharide trimer.
4. Process according to claim 1 or claim 2 wherein the feed solution contains saccharide dimers and 6 wt%-DS or less of saccharide monomers and/or saccharide trimers.
5. Process according to any one of the preceding claims wherein the saccharide dimer is maltose, maltitol or sucrose.
6. Process according to any on of the preceeding claims wherein the saccharide dimer is cellobiose, cellobitol, xyllobiose or xylobitol.
7. Process according to any one of the preceeding claims, wherein the saccharide monomer is glucose, fructose or sorbitol.

8. Process according to any one of the preceding claims wherein the crosslinked cation exchange resin is a strong acid cation exchange resin.

9. Process according to any one of the preceding claims wherein the crosslinked cation exchange resin is a gel type strong acid cation exchange resin.

10. Process according to any one of the preceding claims wherein the saccharides are derived from starch.

11. Process according to claim 10, wherein the saccharides are derived by saccharification of liquefied starch with pullulanase and beta-amylase.

12. Process according to claim 11, wherein the saccharides are derived further by treatment with maltogenic alpha-amylase and subsequent saccharification with low temperature alpha amylase, optionally followed by a final saccharification with maltogenic alpha-amylase.

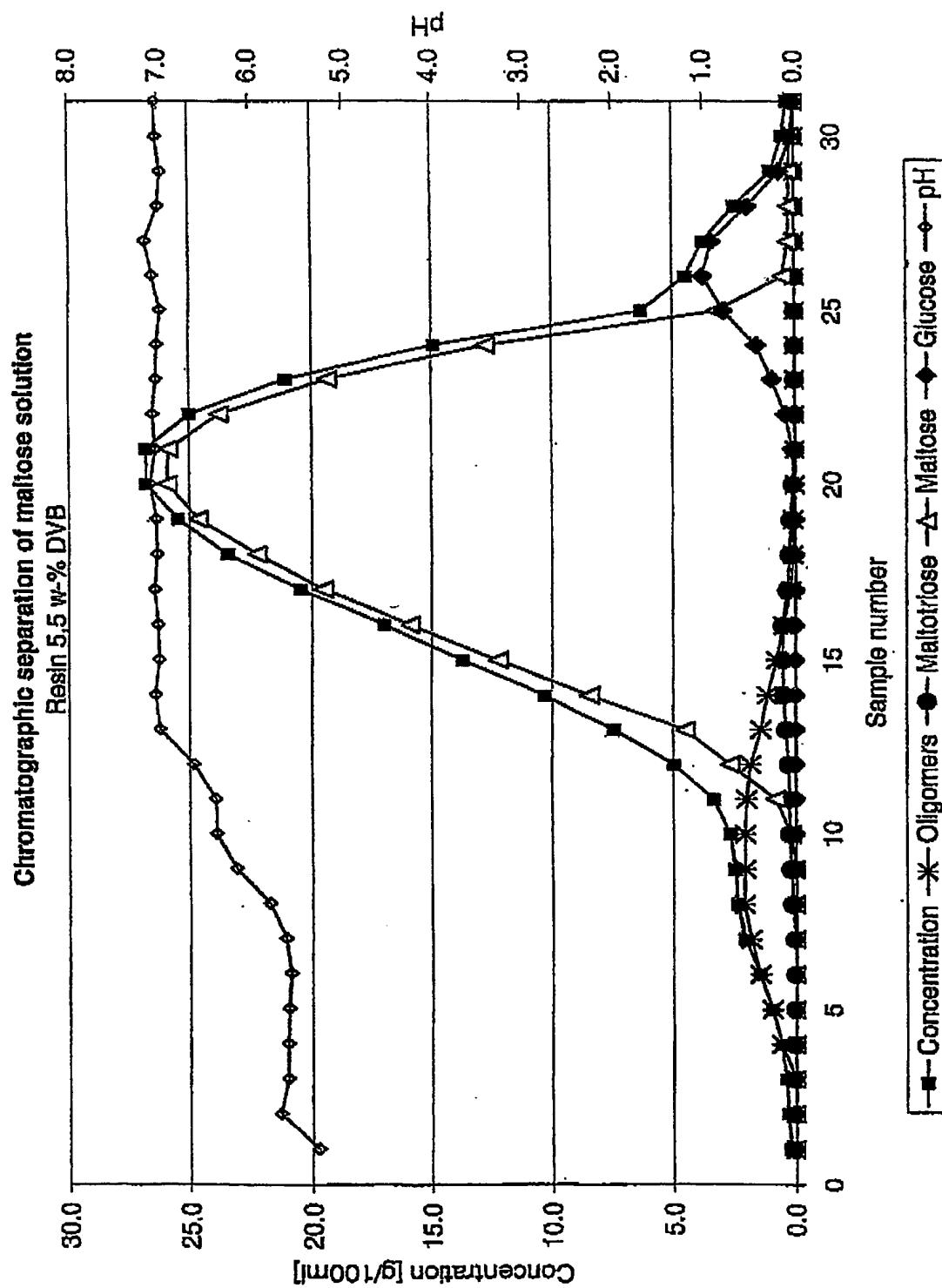
13. Process according to any one of the preceding claims wherein the separation is effected at a temperature of 65 to 90°C.

14. Process according to any one of the preceding claims wherein the separation is effected at a temperature of 80°C or more.

15. Process according to any one of the preceding claims wherein the disaccharide is a sugar alcohol which process comprises the further step of crystallising the sugar alcohol.

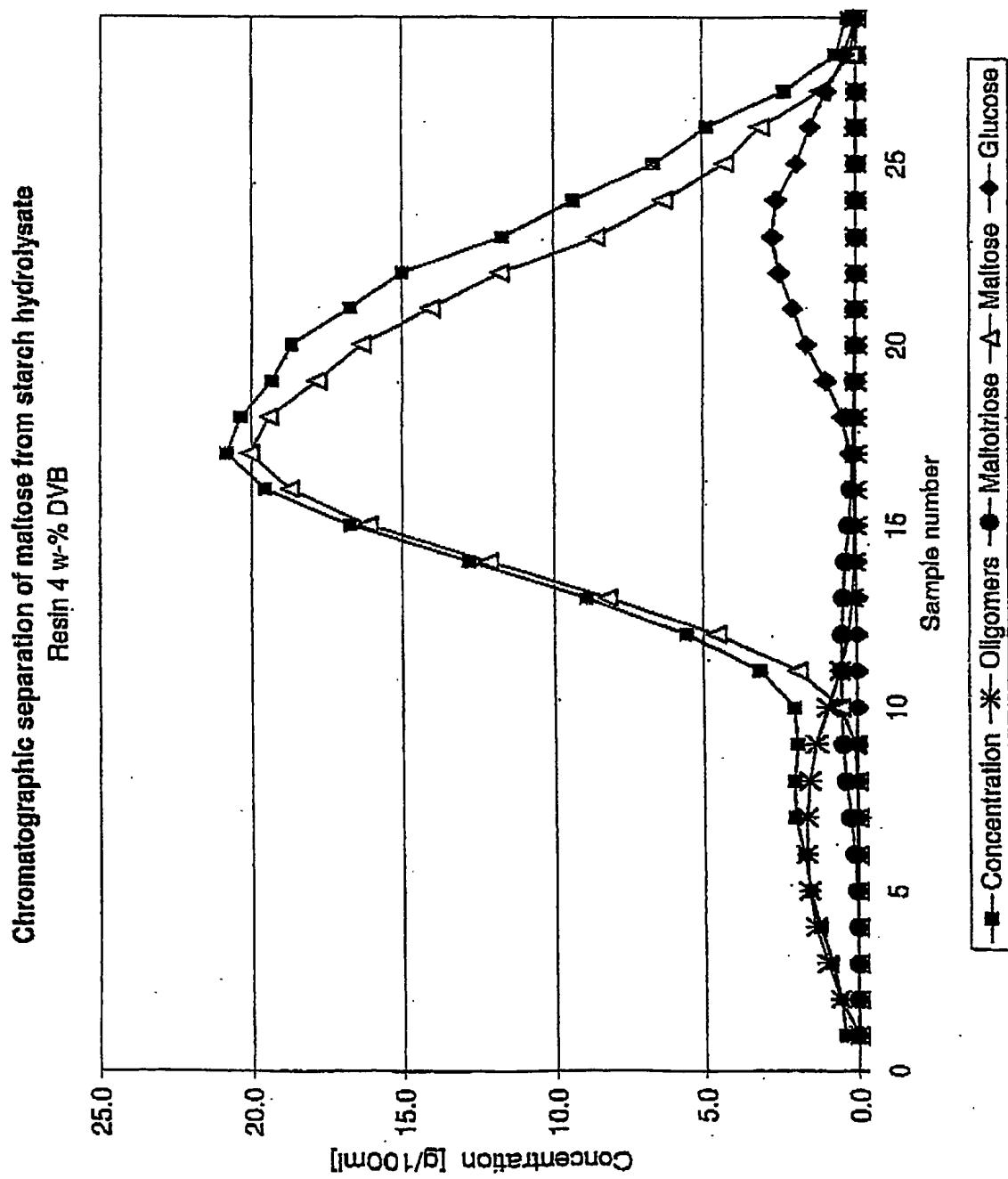
16. Process according to claim 15 wherein the disaccharide sugar alcohol is maltitol.

Fig. 1/2



Best Available Copy

Fig. 2/2



Best Available Copy

## INTERNATIONAL SEARCH REPORT

Inte  
nal Application No  
PCT/EP 03/01091

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C13K1/00	C13K 08	C13K3/00	C13K7/00	C13K11/00
	C13K13/00	C13D1/00	C13D3/00	C13D3/14	C07H1/06
	C07H3/00				

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C13K C13D C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3 817 787 A (AMINOFF C ET AL) 18 June 1974 (1974-06-18) the whole document	1,2,5-9, 13,14
A		3,4,15, 16
Y	---	10-12
X	US 3 864 406 A (MELAJA ASKO J ET AL) 4 February 1975 (1975-02-04) the whole document	1,2,7-9
A		3-6, 10-16
Y	---	
A	US 5 462 864 A (NIIMI MASAHIRO ET AL) 31 October 1995 (1995-10-31) the whole document	1-9, 13-16
Y	---	10-12
A	US 4 325 742 A (ARENA BLAISE J) 20 April 1982 (1982-04-20) the whole document	1-16
	---	
		-/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

## ° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

26 May 2003

04.07.2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

EVA JOHANSSON /EÖ

## INTERNATIONAL SEARCH REPORT

Inte  
nal Application No  
PCT/EP 03/01091

## C.(Continuation) DOCUMENTS CONSIDERED BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 816 445 A (MITSUHASHI MASAKAZU ET AL) 28 March 1989 (1989-03-28) the whole document ---	1-16
A	US 4 487 198 A (MIYAKE TOSHIO ET AL) 11 December 1984 (1984-12-11) the whole document ---	1-16
A	EP 0 098 352 A (UOP INC) 18 January 1984 (1984-01-18) the whole document -----	1-16

## INTERNATIONAL SEARCH REPORT

Inte  
al Application No  
PCT/EP 03/01091

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 3817787	A 18-06-1974	AT 63673 A AU 468579 B2 AU 5116873 A BE 794505 A1 BG 20618 A3 CA 987313 A1 CH 581701 A5 CS 188159 B2 DE 2302841 A1 FI 54609 B FR 2169230 A1 GB 1394503 A HU 166156 B IE 37118 B1 IT 977039 B JP 48082048 A NL 7301178 A ,B, SE 394456 B SU 912046 A3 YU 21773 A1 ZA 7300539 A		15-07-1975 15-01-1976 18-07-1974 16-05-1973 05-12-1975 13-04-1976 15-11-1976 28-02-1979 02-08-1973 29-09-1978 07-09-1973 14-05-1975 28-01-1975 11-05-1977 10-09-1974 02-11-1973 30-07-1973 27-06-1977 07-03-1982 28-02-1982 31-10-1973
US 3864406	A 04-02-1975	AT 339274 A AU 474630 B2 AU 6800474 A CH 605501 A5 CS 187416 B2 DE 2418801 A1 DK 146255 B FI 59085 B FR 2227251 A1 GB 1418450 A HU 169624 B IE 39751 B1 IT 1011279 B JP 1203169 C JP 49134851 A JP 58035169 B NL 7405513 A NO 140183 B PL 102624 B1 SE 405595 B SU 582752 A3 ZA 7402439 A		15-11-1977 29-07-1976 23-10-1975 29-09-1978 31-01-1979 28-11-1974 15-08-1983 27-02-1981 22-11-1974 17-12-1975 28-12-1976 20-12-1978 20-01-1977 25-04-1984 25-12-1974 01-08-1983 29-10-1974 09-04-1979 30-04-1979 18-12-1978 30-11-1977 30-04-1975
US 5462864	A 31-10-1995	JP 2119789 A JP 2696537 B2 US 5141859 A	07-05-1990 14-01-1998 25-08-1992	
US 4325742	A 20-04-1982	NONE		
US 4816445	A 28-03-1989	JP 1866303 C JP 5069090 B JP 61007214 A JP 1866290 C JP 5052177 B JP 61009257 A JP 1834817 C	26-08-1994 30-09-1993 13-01-1986 26-08-1994 04-08-1993 16-01-1986 11-04-1994	

## INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/EP 03/01091

Patent document cited in search report	Publication date		Patent family member(s)		Publication date
US 4816445	A		JP 5043360 B JP 61035800 A JP 1866293 C JP 5077381 B JP 61047166 A AU 582366 B2 AU 4396985 A CA 1265516 A1 CN 85105158 A ,B DE 3522103 A1 DE 3546911 C2 FR 2566409 A1 GB 2162524 A ,B IT 1181701 B KR 9302890 B1		01-07-1993 20-02-1986 26-08-1994 26-10-1993 07-03-1986 23-03-1989 02-01-1986 06-02-1990 31-12-1986 09-01-1986 09-01-1997 27-12-1985 05-02-1986 30-09-1987 12-04-1993
US 4487198	A	11-12-1984	NONE		
EP 0098352	A	18-01-1984	US 4429122 A AT 37042 T DE 3377922 D1 EP 0098352 A2 ES 8503375 A1 JP 58193700 A MX 159946 A		31-01-1984 15-09-1988 13-10-1988 18-01-1984 01-06-1985 11-11-1983 09-10-1989